NORTHERN HYBRIDIZATION

Initially, northern hybridization was carried out exclusively with RNA immobilized on diazotized cellulose (diazobenzyloxymethyl [DBM]-cellulose) (Alwine et al. 1977) (see Chapter 9, pages 9.35–9.36). Subsequently, *o*-aminophenylthioether (APT)-cellulose was developed, which is easier to prepare and more stable than DBM-cellulose (Seed 1982a,b). However, the use of these activated celluloses for immobilization and hybridization of RNA was largely obviated when it was shown that RNA denatured by glyoxal and dimethyl sulfoxide (DMSO) (McMaster and Carmichael 1977), methylmercuric hydroxide (Thomas 1980, 1983), or formaldehyde (Rave et al. 1979; Goldberg 1980; Seed 1982a) binds tightly to nitrocellulose and hybridizes to radioactive probes with high efficiency (White and Bancroft 1982). The sensitivity with which RNA bound to nitrocellulose can be detected by hybridization is such that species of mRNA comprising no more than 0.001% of the mRNA can be rapidly identified and easily quantitated. Large molecules of denatured RNA (>9 kb) transfer from the gel with high efficiency, so that fragmentation of the RNA is generally unnecessary.

The attachment of denatured RNA to nitrocellulose is presumed to be noncovalent but is essentially irreversible. It is therefore possible to hybridize sequentially RNA immobilized on nitrocellulose to a series of radioactive probes without significant loss of the bound nucleic acid. Unfortunately, nitrocellulose filters are not usually durable enough to withstand more than two rounds of hybridization and washing. This difficulty can be solved by transferring the RNA to positively charged nylon membranes, which stand up well to many rounds of hybridization without loss of hybridization signal. However, the background hybridization to many types of nylon membranes is considerably higher than it is to nitrocellulose filters. We therefore recommend that nylon membranes be used only when it is known that the RNA will be hybridized to many probes sequentially. Each manufacturer provides specific instructions for the transfer of nucleic acids to their particular type of charged nylon membrane. These instructions should be followed exactly, since they presumably have been shown to yield the best results. Practical information about the use of nylon membranes can also be found in Chapter 9, pages 9.42–9.43, and in Reed and Mann (1985).

A wide variety of probes may be used to detect RNA transferred to nitrocellulose filters or nylon membranes, including double-stranded DNA labeled by nick translation, single-stranded DNA prepared by primer extension of an oligonucleotide annealed to a recombinant M13 bacteriophage, radiolabeled synthetic oligonucleotides, and RNA synthesized in vitro with prokaryotic DNA-dependent RNA polymerases (e.g., bacteriophage SP6, T7, or T3 RNA polymerase). Methods to synthesize and use these probes are discussed in Chapters 10 and 11.

Below we describe methods for electrophoresis of denatured RNA through agarose gels, for the transfer of the fractionated RNA to nitrocellulose filters and nylon membranes, and for hybridization of the immobilized RNA to radiolabeled probes. The protocol presented for northern hybridization also works well with most types of positively charged nylon membranes but may not be optimal for any particular brand.